Association of *bla*$_{DHA-1}$ and *qnr*B genes carried by broad-host-range plasmids among isolates of *Enterobacteriaceae* at a Spanish hospital

C. Mata[1], E. Miró[1], M. Toleman[2], M. A. Rivera[1], T. R. Walsh[2] and F. Navarro[1]

1) *Departament de Genètica i de Microbiologia, Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Institut d'Investigacions Biomèdiques Sant Pau, Barcelona, Spain and*

2) *Section of Medical Microbiology, IIB, School of Medicine, Cardiff University, Heath Park, Cardiff, United Kingdom*

Abstract

A collection of 30 DHA-1-*Enterobacteriaceae* producers was examined for the presence of *qnr* genes. PCR-based replicon typing, plasmid profile and Southern hybridisation analyses revealed that all isolates co-harboured *bla*$_{DHA-1}$ and *qnr*B genes on the same plasmid. All but one of these plasmids belonged to the L/M group. Genetic organization analyses of a randomly selected isolate revealed the co-localization of both genes on an IS*26*-composite transposon. As plasmids carrying both genes seem to have a high prevalence and a worldwide distribution, care should be taken when quinolones are used to treat infections caused by DHA-1 producers.

Keywords: IncN, IS*26*-composite transposon, L/M incompatibility group, plasmid-mediated AmpC β-lactamases, plasmid-mediated quinolone resistance

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Corresponding author: F. Navarro, Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau, C/Sant Quintí, 89, 08041 Barcelona, Spain
E-mail: fnavarror@santpau.cat

DHA-1, an inducible acquired AmpC enzyme conferring resistance to most β-lactamams, and QnrB, a plasmid-mediated enzyme conferring low resistance to quinolones, have been widely associated with clinical isolates of the family *Enterobacteriaceae* [1,2]. Although these enzymes were first described in 1992 and 2006, respectively [3,4], it was not until some years later that their presence noticeably increased, and they became the most prevalent enzymes of their groups in some countries [1,5,6]. As a strong correlation has been reported between these two resistance mechanisms [6], the aim of this study was to analyse the presence of *qnr* genes on a large characterized collection of DHA-1-*Enterobacteriaceae* producers to determine whether they were localized on the same or different plasmids. As in some cases *bla*$_{DHA-1}$ and *qnr*B genes have been linked together on a complex sul1-type integron [7], we also analysed their genetic organization on the plasmid.

A total of 30 *Enterobacteriaceae* lacking inducible chromosomal *amp*C genes encoding *bla*$_{DHA-1}$ or *bla*$_{DHA-1}$ plus *bla*$_{CTX-M-14,-15}$ were collected from a Spanish hospital (Hospital de la Santa Creu i Sant Pau, Barcelona) from 2005 to 2007 [8]. Bacterial isolates were mainly recovered from urine (76.7%) and most of them (83.3%) were from patients aged 70 to 95 years old. Occurrence of these resistance determinants increased over this period from six cases in 2005 to 16 cases in 2007 [8]. No clonal relationship was established between these isolates except for one cluster of two *E. coli*, one cluster of two *K. pneumoniae* co-producing DHA-1 plus CTX-M-15 and one cluster of two *K. oxytoca* [8].

Using the broth mating method [9], transfer of *bla*$_{DHA-1}$ genes via conjugation was possible in 86.7% (26/30) of the tested strains, with a conjugation frequency ranging from 10^{-5} to 10^{-8} transconjugants per recipient. Transconjugants were selected on LB agar supplemented with ceftazidime (10 mg/L) and rifampin (100 mg/L). Among the five isolates co-harbouring *bla*$_{DHA-1}$ plus *bla*$_{CTX-M14,-15}$ genes, both resistant determinants were transferred in three of them (101-Tc, 103-Tc and 104-Tc) (Table 1).

Antibiotic agents used in the disc diffusion susceptibility test in donor and transconjugant strains are listed in Table 1. All donor and transconjugant strains showed high-level resistance to most β-lactam antibiotic agents, as expected by an *amp*C-producer. Antibiotics displaying higher activity against AmpC β-lactamases were imipenem, cefepime and aztreonam, with percentages of susceptible isolates of 100%, 92.3% and 84.6%, respectively (data not shown). These isolates also showed reduced susceptibility to other antibiotic families (Table 1). Non-β-lactam antibiotics transferred via conjugation were nalidixic acid (61.5%), sulphonamides (30.8%),

TABLE 1. Non-β-lactam resistance profiles in donor and transconjugant strains, location of *bla* and *qnr*B genes and plasmid size

Strains-D/Tc[a]	Species	Isolation data[b]	Non-β-lactam resistance profile[c]		Replicon typing/resistant enzymes/plasmid size (kb)[d]
			Donors	Transconjugants	
35-Tc	*E. coli*	01/05	SSS,SXT, TET, NAL, CIP	SSS	L/M-FIA$^{DHA-1,QnrB}$ (≈171)
36-Tc	*E. coli*	06/05	SSS, TET	NAL	L/M$^{DHA-1,QnrB}$ (≈209)
37-Tc	*E. coli*	07/05	SSS, TMP, SXT, TET, NAL, CIP	SSS, NAL	L/M$^{DHA-1,QnrB}$ (≈100 and 312)[e]
38-Tc	*E. coli*	12/05	SSS, TMP, SXT, NAL, CIP	–	L/M$^{DHA-1,QnrB}$ (≈79)
62-Tc	*E. coli*	06/06	SSS, TMP, SXT, TET, CHL, NAL, CIP	SSS, NAL	L/M$^{DHA-1,QnrB}$ (≈74 and 164)[e]
63-Tc	*E. coli*	07/06	NAL, CIP	NAL	L/M$^{DHA-1,QnrB}$ (≈293)
64-D	*E. coli*	12/06	–	–	F, FIB (lysed)
95-Tc	*E. coli*	01/07	NAL	NAL	L/M$^{DHA-1,QnrB}$ (≈236)
96-Tc	*E. coli*	06/07	SSS, TMP, SXT, NAL, CIP	NAL	L/M$^{DHA-1,QnrB}$ (≈96)
97-Tc	*E. coli*	07/07	SSS, TET, CHL, NAL, CIP	NAL	L/M$^{DHA-1,QnrB}$ (≈173)
98-Tc	*E. coli*	07/07	TET, NAL, CIP	TET, NAL	N$^{DHA-1,QnrB}$ (≈52)
99-Tc	*E. coli*	08/07	SSS, TMP, SXT, TET, NAL, CIP	NAL	L/M-FIA$^{DHA-1,QnrB}$ (≈155)
100-D	*E. coli*	07/07	SSS, TMP, SXT, TET, NAL, CIP	–	L/M, F, FIB (lysed)
101-Tc	*E. coli*	09/07	NAL	NAL	L/M$^{DHA-1,QnrB}$ (≈156), I1/I$\gamma^{CTX-M-14}$ (≈88)
102-Tc	*E. coli*	12/07	SSS, TMP, SXT, TET, NAL, CIP	TMP, TET, NAL	L/M$^{DHA-1,QnrB}$ (≈93), F-FIB (≈148)
40-D	*K. pneumoniae*	01/05	–	–	L/M$^{DHA-1,QnrB}$ (≈79)
41-Tc	*K. pneumoniae*	06/05	NAL	NAL	L/M$^{DHA-1,QnrB}$ (≈202)
66-D	*K. pneumoniae*	08/06	TMP, CHL, NAL	–	L/M$^{DHA-1,QnrB}$ (≈72)
67-Tc	*K. pneumoniae*	10/06	–	–	L/M$^{DHA-1,QnrB}$ (≈72)
103-Tc	*K. pneumoniae*	02/07	TMP, NAL, CIP	TMP, SXT	L/M$^{DHA-1,QnrB,CTX-M-15}$ (≈168)
104-Tc	*K. pneumoniae*	03/07	TMP, NAL, CIP	TMP	L/M-FIC$^{DHA-1,QnrB,CTX-M-15}$ (≈153)
105-Tc	*K. pneumoniae*	06/07	SSS, TMP, SXT, TET, CHL, NAL, CIP	–	L/M$^{DHA-1,QnrB}$ (≈72)
106-Tc	*K. pneumoniae*	06/07	NAL	NAL	L/M$^{DHA-1,QnrB}$ (≈72)
107-Tc	*K. pneumoniae*	08/07	NAL, CIP	–	L/M$^{DHA-1,QnrB}$ (≈72)
108-Tc	*K. pneumoniae*	10/07	SSS, TMP, SXT, TET, CHL, NAL, CIP	–	L/M$^{DHA-1,QnrB}$ (≈70)
68-Tc	*K. oxytoca*	11/06	–	–	L/M$^{DHA-1,QnrB}$ (≈72)
69-Tc	*K. oxytoca*	12/06	SSS, TMP, SXT, TET	–	L/M$^{DHA-1,QnrB}$ (≈72)
109-Tc	*K. oxytoca*	06/07	SSS, TMP, SXT, TET, NAL, CIP	NAL	L/M-FIC$^{DHA-1,QnrB}$ (≈153)
110-Tc	*K. oxytoca*	08/07	NAL	NAL	L/M$^{DHA-1,QnrB}$ (≈112)
77-Tc	*P. mirabilis*	10/06	SSS, TMP, SXT, TET, NAL, CIP	SSS, TMP, SXT, TET, NAL	L/M-N$^{DHA-1,QnrB}$ (≈103)

Ampicillin, piperacillin, cephalotin, cefuroxime, cefotaxime, ceftazidime, cefepime, aztreonam, cefoxitin, amoxicillin-clavulanic acid, piperacillin-tazobactam and imipenem were also used for susceptibility testing. As the recipient used for conjugation was resistant to aminoglycoside antibiotics, this antibiotic family was not included in the study.
NAL, nalidixic acid; CIP, ciprofloxacin; SSS, sulphonamides; TMP, trimethoprim; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol.
[a]Names assigned to the strains. D, donor strains due to the inability to obtain transconjugants; Tc, transconjugant strains.
[b]Isolation date of donor strains (month/year).
[c]The disc diffusion susceptibility test was performed according to Clinical Laboratory Standards Institute (CLSI) guidelines. All transconjugant strains resistant to NAL displayed reduced susceptibility (14–18 mm; intermediate as clinical category).
[d]Plasmid analyses results given are from transconjugant strains with the exception of 40-D, 64-D, 66-D and 100-D due to the inability to obtain transconjugants in these isolates.
[e]bla_{DHA-1}, L/M replicon and *qnr*B probes hybridized in both plasmids present in this isolate.

trimethoprim (15.4%), tetracycline (11.5%) and trimethoprim-sulphamethoxazole (7.7%) (Table 1).

Screening of *qnr* genes in all 30 isolates was carried out by PCR amplification [10]. *qnrB* genes were present in all isolates tested. These results were in agreement with other reports that found a close association between *qnr*B, especially *qnr*B4, and bla_{DHA-1} determinants in isolates of the family *Enterobacteriaceae* [6,11–13].

PCR-based replicon typing, plasmid profile and Southern hybridization analyses were performed in all 30 DHA-1 producers (Table 1) as previously described [9]. Two of these isolates were lysed during the S1-PFGE and therefore could not be analysed. To determine whether both resistance genes were localized on the same or different plasmids, hybridization with bla_{DHA-1} and *qnr*B probes was performed. Results revealed the co-localization of bla_{DHA-1} and *qnr*B resistance genes on the same conjugative plasmid in all 28 isolates tested (Table 1). All but one of the plasmids associated with *qnr*B, bla_{DHA-1} or bla_{DHA-1} plus bla_{ESBL} belonged to the broad-host-range L/M plasmids (Table 1), while bla_{DHA-1} has traditionally been found on narrow-host-range IncFII plasmids [14]. The single isolate not linked to the IncL/M group, carried both resistance genes on an IncN plasmid. This is the first time that both bla_{DHA-1} and *qnr*B genes have been described on an IncN plasmid [14]. The new localization of bla_{DHA-1} and *qnr*B4 genes in broad-host-range L/M plasmids has been postulated as a possible cause of the lately widespread distribution of bla_{DHA-1} genes [9]. Our results reinforce this idea, together with the fact that a strong association between *qnr*B and bla_{DHA-1} has been evidenced worldwide [2,6,7,15] and that recent reports have found bla_{DHA-1} and *qnr*B genes also in L/M plasmids [16].

The genetic organization of these 30 isolates carrying bla_{DHA-1} has been previously analysed by overlapping PCR amplification, exploring the most frequent regions surrounding *amp*C genes described in the literature (data not shown). As 93.3% (28/30) of the isolates displayed the same genetic organization, an IS*26* element and a region from the *Morganella morganii* genome found upstream and downstream of bla_{DHA-1}, respectively, one of them was randomly selected

for further analyses. Plasmid extraction of this *E. coli* isolate was performed. Isolated plasmids were sequenced by Eurofins MWG Operon using GS FLX Titanium series chemistry technology. Partial analysis of the sequenced plasmid, called pEC37Tc, showed that bla_{DHA-1} and *qnr*B4 were localized together on an IS*26*-composite transposon, most likely involved in their mobilization (Fig. 1). Other non-resistance genes found in this structure were the 3′CS extreme of a class 1 integron and the stress-inducible psp (phage shock protein) operon (Fig. 1). Under a wide variety of environmental signals, the psp operon is highly transcribed to conserve membrane integrity, efficient translocation and maintenance of the proton motive force [17]. This structure, carrying beneficial traits to the bacteria such as antibiotic resistance genes and a wide response to environmental factors, may contribute to the maintenance of the plasmid in the bacteria. Linkage of bla_{DHA-1} and *qnr*B4 genes on similar structures has so far been described in isolates of *Klebsiella* spp, mainly *K. pneumoniae* [7,18,19]. To our knowledge, this is the first time that this genetic organization is described in an *E. coli* strain.

The presence of *qnr* genes has been associated with an increase in the quinolone MIC values and treatment failures when quinolones are used to treat quinolone-susceptible enterobacteria [15]. As plasmids carrying both resistance determinants seem to have a high prevalence and a worldwide distribution, care should be taken when quinolones are used to treat infections caused by DHA-1-*Enterobacteriaceae* producers.

Nucleotide Sequence Accession Number

The GenBank accession number of the sequence presented here is HQ700359 (pEC37Tc).

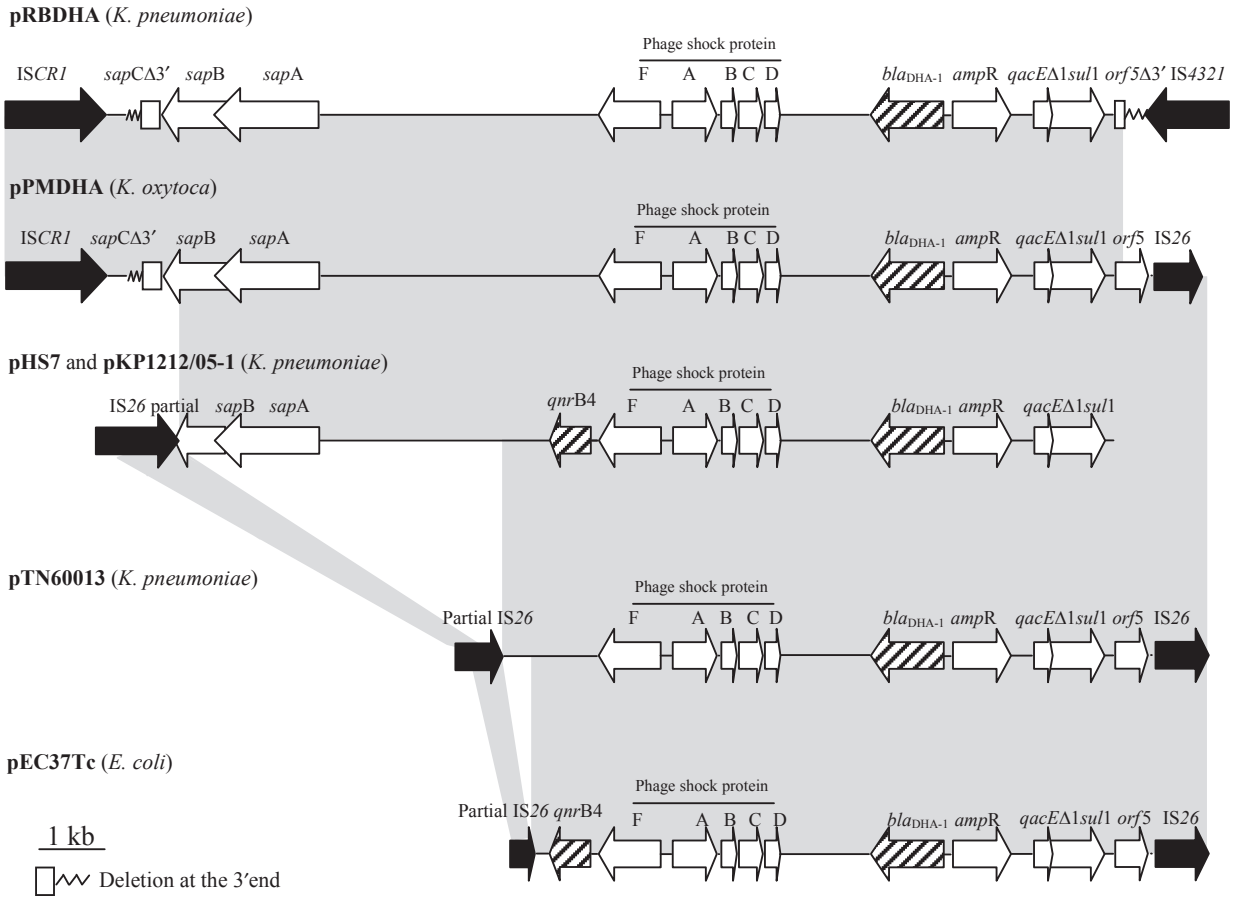

FIG. 1 Genetic organization of pEC37Tc in comparison with the common regions of other plasmids carrying bla_{DHA-1} and *qnr*B genes. GenBank accession numbers of pRBDHA, pMPDHA, pHS7, pKP1212/05-1, pTN60013 and pEC37Tc are AJ971343, AJ971344, EF683583, FJ943500, AJ971345 and HQ700359, respectively. Genes are represented by arrows. The resistant genes *qnr*B4 and bla_{DHA-1} are represented by filled arrows. Insertion sequences are represented by black arrows. Shaded regions are regions of 100% identity. Although *qnr*B4 genes are present in all the plasmids shown, the arrow for this gene is not drawn in pRBDHA, pMPDHA and pTN60013 because it is not annotated in the submitted GenBank sequence.

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Transparency Declaration

The authors declare that they have no conflict of interest.

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Enhanced isolation of *Legionella* species from composted material

S. McCabe, A. Brown, G. F. S. Edwards and D. Lindsay

Scottish Haemophilus Legionella Meningococcus Pneumococcus Reference Laboratory (SHLMPRL), House on the Hill, Stobhill Hospital, Glasgow, UK

Abstract

Legionella pneumophila and *Legionella* species were isolated from composted material when freshly prepared buffered charcoal yeast extract (BCYE) was supplemented with glycine (1.5 g/L), polymyxin B sulfate (40 000 IU/L), vancomycin hydrochloride (0.5 mg/L) and cycloheximide (40 mg/L) (GVPC medium) and Modified Wadowsky–Yee (MWY) (Oxoid, Cambridge, UK) plates were used for cultivation, but not with commercially sourced pre-poured GVPC and MWY plates (Oxoid). *Legionella cincinnatiensis* and pathogenic *L. pneumophila* serogroup (Sg) 1 Benidorm and France/Allentown were identified, as well as a non-typeable (NT) strain of *L. pneumophila*. As most laboratories no longer produce their own media, this may contribute to the lack of positive cultures from composted material. The antigenicity of the NT strain is discussed.